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**THE EFFECTS OF ACUTE BEETROOT JUICE  
SUPPLEMENTATION ON THE PHYSIOLOGICAL  
RESPONSES DURING A RUGBY LEAGUE MATCH  
SIMULATION PROTOCOL AND RECOVERY**

**by**

**Casey Walker**

A Research Project submitted in partial fulfilment of the  
requirements of the University of Chester for the degree of  
M.Sc. Sports Sciences (Physiology Pathway)

September, 2014

No portion of the work referred to in this Research Project has been submitted in support of an application for another degree or qualification of this, or any other University or institute of learning.

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## Abstract

The purpose of this investigation was to examine the physiological effects of acute BR supplementation on rugby league specific intermittent running performance, while also examining the effects of further supplementation on recovery from EIMD. It was hypothesized that BR supplementation would lead to improved intermittent performance and promote recovery from a rugby league match simulation protocol (RLMSP). An experimental, randomized, independent groups design was adopted, including one control group ( $n = 6$ ) and one experimental group ( $n = 6$ ). A 70 mL beetroot juice drink (BR) ( $\sim 6.5$  mMol nitrate; 697.9  $\mu$ mol total antioxidant capacity) or control beverage was consumed 2.5 h prior to RLMSP performance. Participants were then required to ingest further beverages, twice a day, separated by 6-8 h for 48 h following the RLMSP. A 2-way (group [2] x time [4]) analysis of variance, independent and paired sample  $t$ -tests were conducted. Relative peak sprint speed was maintained in quartile 4 of the RLMSP in the BR group ( $p > 0.05$ ). There was no significant difference between groups for mean speed and high speed running ( $p > 0.05$ ). Blood glucose significantly decreased ( $p < 0.05$ ) in the BR group and not the control. No differences were found for oxygen consumption, blood lactate, heart rate and RPE ( $p > 0.05$ ). BR attenuated EIMD and promoted recovery from the RLMSP evidenced by attenuated CK accumulation, maintained neuromuscular function and a reduced increase in perceived muscular soreness ( $p < 0.05$ ). The findings of this study suggest that acute BR supplementation may enhance intermittent performance and attenuate EIMD following rugby league specific exercise.

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## 1. Chapter 1 - Introduction

Rugby league, played over two 40 min halves, is an intermittent contact sport with predominant periods of low intensity activity (walking, standing and jogging) interspersed with high-intensity movements (sprinting and high-speed running). Rugby league players, depending upon position, can complete distances of up to  $8503 \pm 631$  m during a competitive game (Sykes, Twist, Hall, Nicholas, & Lamb, 2009; Meir, Arthur & Forrest, 1993; Gabbett, King & Jenkins, 2008). Due to large distances covered and intermittent nature of a rugby league match, both the aerobic and anaerobic energy systems are heavily taxed, often leading to fatigue, an example of which may be reflected by a decline in high-intensity running or sprint speeds during the latter stages of a match (Waldron, Highton, Daniels & Twist, 2013; Gabbett, 2005). This was evidenced by Sykes et al. (2011), where professional players locomotive rates at very high intensities ( $>19.8 \text{ km}\cdot\text{h}^{-1}$ ) were  $3.8 \text{ m}\cdot\text{min}^{-1}$  slower in the fourth quartile of a match than in the first. Due to rugby leagues international recognition, enhancing performance and recovery from matches in order to facilitate success has received an abundance of attention from sports science practitioners in academic literature (Kempton, Sirotic & Coutts, 2014; Twist et al., 2012; McLellan et al., 2011; Gabbett, 2005; Gill, Beavan & Cook, 2006).

Recent studies have investigated the ergogenic properties of nitric oxide (NO), found in beetroot juice (BR), while some authors have also speculated that BR may possess recovery benefits (Ormsbee, Lox & Arciero, 2013; Cermak et al., 2012; Wylie et al., 2013; Lansley et al., 2012). Studies have reported that dietary nitrate, naturally found in BR is converted into nitrite

and finally NO, believed to be an ergogenic compound. NO has been a popular area of research in recent years with improvements found in walking, running, cycling, rowing and during intermittent exercise (Bailey et al., 2010; Bailey et al., 2009; Larsen et al., 2007; Lansley et al., 2011; Larsen et al., 2010; Vanhatalo et al., 2010a; Wylie et al., 2012). Improvements in exercise performance have been attributed to various mechanisms including reductions in whole body oxygen consumption ( $\dot{V}O_2$ ) due to improvements in mitochondrial respiration, improved oxygen delivery mediated by vasodilation and an improved energy cost of force production through the regulation of calcium release (Barclay, Woledge & Curtin, 2007).

Bailey et al. (2009) were the first to examine the ergogenic effects of NO supplementation. A 16% improvement in time to task failure during a cycling protocol was found following 6 days of NO supplementation (Bailey et al., 2009). Lansley et al. (2011b), adopting a more ecologically valid time-trial protocol found that acute (2.5 h prior) dietary nitrate supplementation resulted in improvements of 2.8% and 2.7% in 4 and 16.1 km cycling time trial performance. The findings from this study suggest that an acute bolus of BR can have meaningful real world improvements on performance, however, this study does not elucidate the effects of BR on intermittent performance.

Few studies have considered the effects of dietary nitrate during intermittent exercise. Wylie et al. (2013) found that following 2-day BR supplementation, performance was significantly improved in the Yo-Yo intermittent recovery level 1 (Yo-YoIR1) by 4.2%. The authors speculated that improved performance was mediated by improved glucose uptake, improved

potassium handling and enhanced maintenance of muscle excitability via the regulation of calcium release. The Yo-YoIR1 however, is not specific to the match demands of rugby league; a more ecologically valid test would be one replicating the time-motion demands of rugby league.

Markers of exercise-induced muscle damage (EIMD), including increased creatine kinase, perceptual soreness and reduced muscular function have been found following intermittent exercise, specifically following professional rugby league matchplay (Twist et al., 2012; McLellan et al., 2011). Indeed, Twist & Sykes (2011) found significantly reduced peak knee extensor torque at  $60 \text{ deg} \cdot \text{s}^{-1}$  for up to 48 h (reductions of  $\sim 40 \text{ N}\cdot\text{m}$  at 0 h,  $\sim 30 \text{ N}\cdot\text{m}$  at 24 h,  $\sim 10 \text{ N}\cdot\text{m}$  at 48 h), significant reductions in countermovement jump performance at 0 and 24 h ( $\sim 3$  and  $\sim 2 \text{ cm}$ , respectively) and significantly increased blood creatine kinase (CK) concentration at 24 h ( $300 \text{ U}\cdot\text{L}^{-1}$ ) following a rugby league match simulation protocol (RLMSP).

EIMD on a cellular level is characterized by ultrastructural changes of myocytes, including z-band streaming, damaged t-tubules, sarcoplasmic reticulum and general sarcomere disruption, leading to impaired excitation-contraction coupling and consequently, muscular contraction (Byrne, Twist & Eston, 2004). The aetiology of EIMD is not fully understood, however, EIMD commonly occurs following eccentric muscular contraction where high forces are generated with little muscle fibre recruitment, placing high mechanical stress on muscle sarcomeres (Byrne, Twist & Eston, 2004). EIMD also occurs metabolically, this form of damage is due to an exercise-induced increase in oxygen consumption leading to a 200-fold increase in oxygen usage in the mitochondria (Paternelj & Coombes, 2011). Radical oxygen species (ROS)

are formed when oxygen is unable to bind to hydrogen following oxidative phosphorylation in the mitochondria (Paternelj & Coombes, 2011). ROS production reportedly induces muscle cell damage through the oxidization of intracellular proteins and membranes, leading to protein leakage, muscle cell death, disturbed genetic transcription and ultimately, impaired muscle function (Barreiro & Hussain, 2010; Staib, Tümer & Powers, 2009; Thiebaud, 2012; Tee, Bosch & Lambert, 2007).

There is evidence that antioxidant supplementation can attenuate EIMD by donating electrons to oxidize ROS, and/or scavenging free radical intermediates associated with EIMD (Paterneli & Coombes, 2011; Silva et al., 2010;). However, studies on antioxidant supplementation have looked at oxidative stress markers such as CK, with little research on muscle function (Jakeman & Maxwell, 2007; Silva et al., 2010). In addition, the evidence surrounding antioxidants is less than conclusive, with some studies finding little to no benefits (Beaton et al., 2002) and others suggesting antioxidants may promote muscle damage and hinder cell adaptation following exercise (Childs et al., 2001; Bryant et al., 2003; Gomez-Cabrera Domenech & Vena, 2008).

NO is an effective vasodilator that is manufactured within the parts of the muscle that are receiving less, or expending more, oxygen (Bailey et al., 2009; Bescós et al., 2012). This mechanism can help to match local blood flow to the parts of the muscle requiring more oxygen, providing a more uniform delivery of nutrient rich blood to the active skeletal muscle and (Bailey et al., 2009). Somewhat paradoxically however, reperfusion or enhanced

blood flow to damaged muscle cells could also cause an enhanced inflammatory response, potentially further damaging muscle architecture and delaying recovery (Tidball, 2005; Thiebaud, 2012). This study can contribute to the body of literature, indicating what mechanism has the largest influence on recovery up to 48 h following exercise.

Therefore, the purpose of this investigation is to examine the physiological effects of acute BR supplementation on rugby league specific intermittent running performance, while also examining the effects of further supplementation on EIMD and recovery. It is hypothesized that acute BR supplementation will improve mean speed, sprint speeds, high-intensity intermittent running performance and attenuate symptoms of EIMD, including neuromuscular function, perceptual soreness and attenuate CK leakage in the 48 h following exercise.

## 2. Chapter 2 - Method

### 2.1. Participants

Following approval from the Faculty of Applied and Health Sciences Research Ethics Committee (appendix 1), 12 healthy male participants aged 18-25 were recruited (stature,  $178.5 \pm 6.03$ ; mass,  $78.6 \pm 8.26$ ; predicted  $\dot{V}O_{2\text{MAX}}$ ,  $54.1 \pm 6.25$ ). Using G\*Power software (Version 3.1 for Mac), a sample size of 12 (two groups of six) was determined as sufficient (appendix 15). Participants were excluded from the study if they did not reach level 9 of the 20 m multistage fitness test (predicted maximal aerobic power  $>45 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), representing the average aerobic fitness of a rugby league player (Gabbett, King & Jenkins, 2008).

### 2.2. Design

The study adopted an experimental, randomized, independent groups design, including one control group and one experimental group. Participants will be required to complete a total of 4 visits during this study (see figure 1). On the first testing day, participants were asked to arrive at 10:00 a.m, following an overnight fast. Participants allocated to the control group were required to consume a blackcurrant drink (16g carbohydrate, 2.5g protein) or a 70 mL nitrate concentrated BR shot (James White Drinks, Ipswich, UK;  $\sim 6.5 \text{ mMol}$  nitrate; total antioxidant capacity of  $697.9 \text{ umol}$ ), 2.5 hrs prior to visit 2. Participants were asked to consume one concentrated BR or control beverage 6 h following the RLMSP. Participants were then asked to consume a BR or control beverage 2.5 h prior to returning to the lab on test day 3,



followed by an additional beverage 6-8 h later. Participants were then asked to consume one further beverage 2.5 h prior to returning to the lab on test day 4 (see figure 1) in order to maintain circulating plasma nitrate (Webb et al., 2008). Testing took place over a 10-day period with a 7-day nitrate and antioxidant intake restriction between visit 1 and 2.

Participants were asked to abstain from strenuous exercise, alcohol, foods rich in dietary nitrate (appendix 2) including beetroot, spinach and other leafy greens, foods with a high antioxidant content (appendix 3) and complete a 10-day food diary (appendix 4). Athletes were requested to follow a diet incorporating a moderate-high carbohydrate intake (60–70% of energy consumed,  $\sim 5\text{--}7\text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ), protein consumption of  $\sim 1.5\text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  and fat ingestion of  $\sim 10\text{--}20\%$  of their whole energy consumption (Appendix 5). Participants were asked to sustain an elevated carbohydrate ( $1\text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) and protein ( $0.3\text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) ingestion for 2 h following the RLMSP in accordance with the practice of elite rugby league players (Twist et al., 2012) (Appendix 6). In addition, participants were required to refrain from using antibacterial mouthwash and chewing gum during the supplementation periods as these have been found to eradicate the oral bacteria necessary for the conversion of nitrite to nitrate (Govoni, Jansson, Weitzberg & Lundberg, 2008).

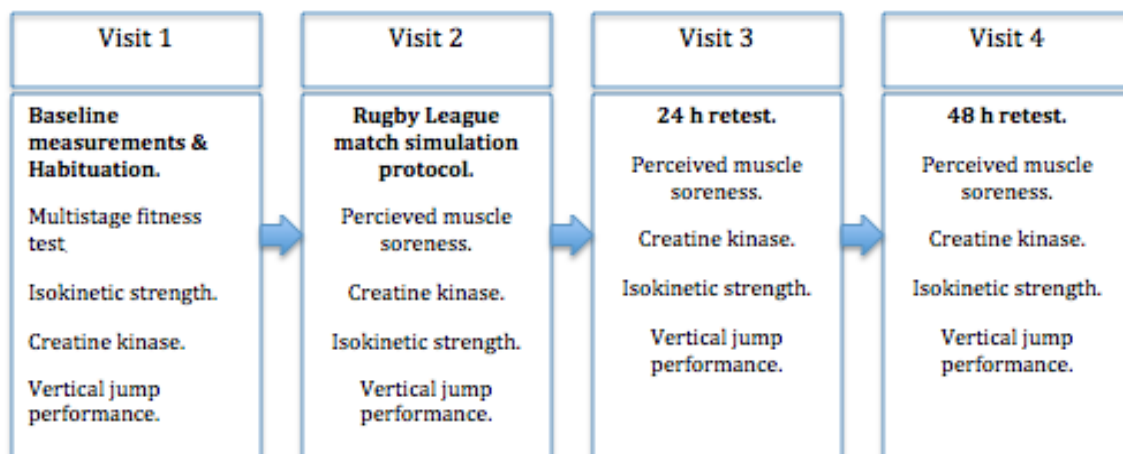


Figure 1. Schematic of study design

### 2.3. Procedures

Participants were required to complete a series of baseline measurements and familiarization tasks (figure 1), including one block of the RLMSP, during visit 1. Familiarization concluded once the participant was familiar with the procedures producing consistent results on the equipment (peak power on the isokinetic dynamometer and vertical jump). Following familiarization the participants completed the multistage fitness test according to the procedure described by Léger, Mercier, Gadoury & Lambert (1988).

#### 2.4. RLMSP

Following baseline measurements each participant was required to complete the RLMSP after completion of a 10 min standardized warm-up, performed outdoors on an artificial pitch. Humidity was 28% and temperature was 26.35 °C. The RLMSP was split into four quartiles lasting a total of 92.08 min, with a 10-min passive recovery following quartile 2, replicating a half-time period. Participants were allowed to ingest water *ad libitum* at the end of each quartile. Participants' locomotive speeds were dictated by an audio CD, with changes being signalled by a “beep” and an instructive voice command. The RLMSP-i consisted of 48 cycles and included a total of 96 sprints. The RLMSP-i (proved reliable to detect moderate changes in performance by Waldron, Highton & Twist, 2013) test was selected and repeated 4 times in order to replicate a worst case scenario in terms of distance covered in a Rugby League match (Meir, Arthur & Forrest, 1993; Gabbett, King & Jenkins, 2008). The test was also adapted to elicit maximal muscle damage, mechanically through eccentric muscle contraction due to 96 sprint decelerations and metabolically due to the extended exercise duration.

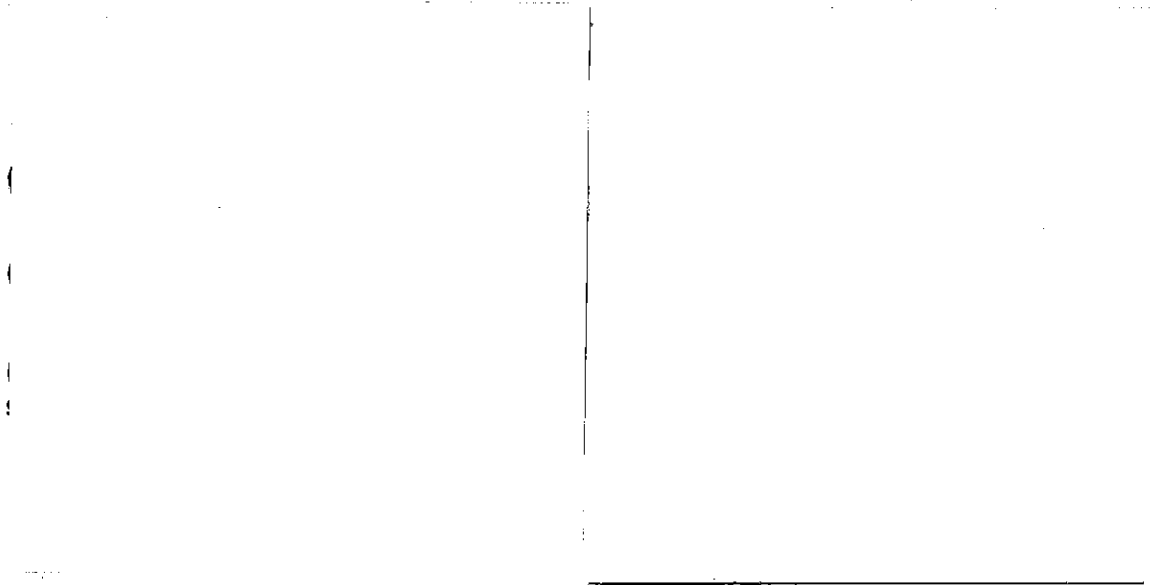


Figure 2. Schematic representation of the layout of the testing area and locomotor instructions used for the rugby league match simulation protocol. Taken from Waldron, Highton & Twist. (2013). Y, yellow cone; R, red cone; B, blue cone; W, white cone.

### *2.5. GPS and heart rate*

Each participant wore a 10 Hz non-differential global positioning system (Catapult Optimeye S5, Catapult Innovations, Melbourne, Australia) positioned between the scapulae, and integrated heart rate monitor (Polar Electro, Oy, Finland) to measure individual movement patterns such as peak sprint speeds, mean quartile speed and high speed running (classifying high speed running  $> 17 \text{ km}\cdot\text{h}^{-1}$ ). Peak sprint speeds were made relative to each participant's first sprint in order to account for differences absolute sprint speeds. GPS & heart rate data was examined using software provided by Catapult Innovations (Catapult Innovations, Melbourne, Australia).

## *2.6. Oxygen consumption*

Participants wore a portable online gas analyser (K4 b2, Cosmed, Cosmed S.r.l., Rome, Italy) to measure oxygen consumption throughout the RLSMP-i4. To facilitate the use of the portable online gas analyser during the RLMSP, the contact simulation phase of the protocol was adjusted so that participants completed a shoulder width press-up. Due to technical errors with the portable online gas analyzer, oxygen consumption data was lost for 2 participants in the control group.

## *2.7. Blood measurements*

Blood glucose (Accu-Check Aviva, Roche Diagnostics, Mannheim, Germany) and lactate (Lactate Pro, Arkray, Kyoto, Japan) were taken via fingertip capillary sample, before, at the halfway stage and immediately following the RLMSP. Creatine kinase activity was assessed at baseline, immediately, 24 and 48 h following the RLMSP via fingertip capillary sample and analysed using a colorimetric assay procedure (Reflotron, Boehringer Mannheim, Germany).

## *2.8. Blood pressure, height and body mass*

Following standardized procedures blood pressure of the brachial artery was measured on all test days, with subjects in a seated position using an automated sphygmomanometer (Dinamap Pro; GE Medical Systems, Tampa, FL). Participants' height using a stadiometer (Wall mounted, Harpenden, Holtain, Crymych, Dyfed, UK) and body mass (BWB-800, Tanita, Tanita Corporation, Tokyo, Japan) were also measured on each test-day.

### *2.9. Isokinetic strength*

Participants performed a warm-up consisting of 5-min cycling at 100 W (model E834, Monark, Vardup, Sweden) before measuring isokinetic strength. Peak knee extensor and flexor torques were measured at 60 and 240 deg·s<sup>-1</sup> using the dominant limb on an isokinetic dynamometer (Biodex 3, Biodex Medical Systems, Shirley, NY, USA). The dominant leg was attached to the input arm of the dynamometer, limb mass measured by the dynamometer to allow for gravitational correction of peak torques and the range of motion determined by the researcher. After two rehearsals, participants performed five maximal efforts at each velocity with a 2 min rest between sets. The highest torque achieved at each velocity was then be used for analysis.

### *2.10. Perceived muscle soreness*

Participants were asked to indicate a level of perceived muscle soreness for the lower limbs using a visual analogue sliding scale. The scale was numbered on the opposite-side; 0 indicated “no soreness on movement”, 5 indicated “muscles sore on movement”, and 10 indicated that the “muscles were too sore to move”. Using procedures validated by Price, McGrath, Rafli & Buckingham (1983), participants were asked to squat with hands on hips and knees shoulder-width apart to a knee joint angle of approximately 90° and rate their level of perceived soreness.

### *2.11. Vertical jump*

Before, immediately, 24 h and 48 h following the RLMSP, participants were required to perform three countermovement jumps with the highest jump

being taken for analysis. A countermovement jump commenced with the participant in an upright position after which they will be asked to rapidly flex the knees to roughly 90° before jumping for maximal height. Flight time was recorded using an infrared timing system (Optojump, Microgate S.r.l, Bolzano, Italy) connected to a computer where flight time was calculated.

## *2.12. Statistical analysis*

Data were analysed using the statistical package for social sciences for Mac (Version 21.0, IBM, Armonk, NY, USA). Descriptive variables were generated with data expressed as the mean  $\pm$  standard deviations. All data was normally distributed with assumptions for normality assessed using the Shapiro-Wilk statistic (sample size <50). Between groups differences in oxygen consumption, mean movement speed, heart rate and blood pressure will be analysed using independent *t*-tests. Differences in creatine kinase activity, potassium concentration, vertical jump performance and isokinetic strength from baseline, immediately, 24 and 48 h and perceived muscle soreness immediately, 24 and 48 h following the RLMSP, were analysed using a 2-way (group [2] x time [4]) repeated measures (analysis of variance) ANOVAs. Changes in blood glucose, potassium and lactate were analysed using a 2-way (group [2] x time [4]) repeated measures ANOVA. Assumptions of sphericity were assessed using the Mauchly test of sphericity, with any violations adjusted by the use of the Greenhouse-Geisser correction. When significance effects were observed, post-hoc paired *t*-test analysis with a Bonferroni correction were performed to determine the location of differences. Statistical significance was set at  $p < 0.05$  for all analysis.

### 3. Chapter 3 - Results

#### 3.1. Mean speed in each quartile

ANOVA revealed no time effect ( $F_{3, 30} = 2.418$ ,  $p > 0.05$ ) or group x time interaction ( $F_{3, 30} = 1.622$ ,  $p > 0.05$ ) on mean speed in each quartile. The control group's locomotor speed in each quartile was  $5.56 \pm 0.49$ ,  $5.63 \pm 0.49$ ,  $5.5 \pm 0.48$  and  $5.4 \pm 0.4$  km·h<sup>-1</sup> in quartiles 1, 2, 3 and 4, respectively. The BR groups locomotor speed in each quartile were  $5.2 \pm 0.53$ ,  $5.02 \pm 0.48$ ,  $5.03 \pm 0.38$  and  $5.03 \pm 0.38$  km·h<sup>-1</sup>, in quartiles 1, 2, 3 and 4, respectively.



### 3.2. Mean relative sprint speed in each quartile

There was no group x time interaction ( $F_{3, 30} = 0.638$ ,  $p > 0.05$ ) on mean relative sprint speed in each quartile, however, a significant time effect was discovered ( $F_{3, 30} = 11.168$ ,  $p < 0.05$ ) (figure 3).

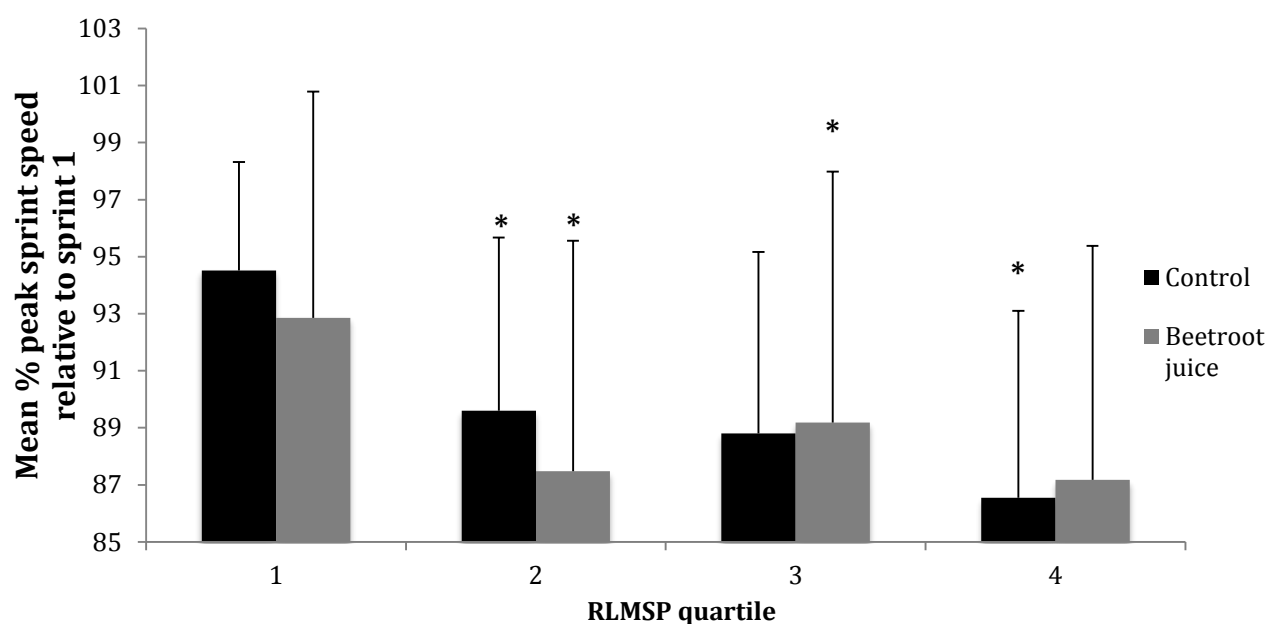


Figure 3. Mean sprint speed in each quartile relative to sprint 1. \* denotes a significant difference to quartile 1 ( $p < 0.05$ ). Data expressed as means  $\pm$  standard deviations.

### 3.3. High-speed running

There was no significant difference in high speed running between groups ( $t = 0.47$ ,  $p > 0.05$ ). The control group performed high speed running for  $5.41 \pm 1.95$  % of the RLMSP whereas the BR group performed high speed running for  $4.63 \pm 3.18$ %.

### 3.4. Blood Pressure

Systolic blood pressure was not significantly decreased, however, there was a significant decrease in diastolic blood pressure following BR supplementation (table 1).

Table 1. Changes in blood pressure following supplementation of BR or placebo.

Group	Baseline diastolic blood pressure	Diastolic blood pressure following supplementation	Baseline systolic blood pressure	Systolic blood pressure following supplementation
<b>Control</b> (mmHg)	66.17 ± 3.44	66.67 ± 3.73	125.67 ± 2.56	124.5 ± 4.11
<b>BR</b> (mmHg)	121.17 ± 2.87	60.5 ± 1.1*	125.33 ± 3.76	121.17 ± 2.87

---

\* denotes a significant difference to baseline ( $p < 0.05$ ).

### 3.5. Blood glucose

ANOVA revealed blood glucose significantly changed over time during the protocol ( $F_{4, 40} = 15.8$ ,  $p < 0.05$ ), with reductions evident from the second quartile of the protocol onwards in the BR group (figure 3). No group x time interaction was apparent ( $F_{4, 40} = 2.33$ ,  $p > 0.05$ ).

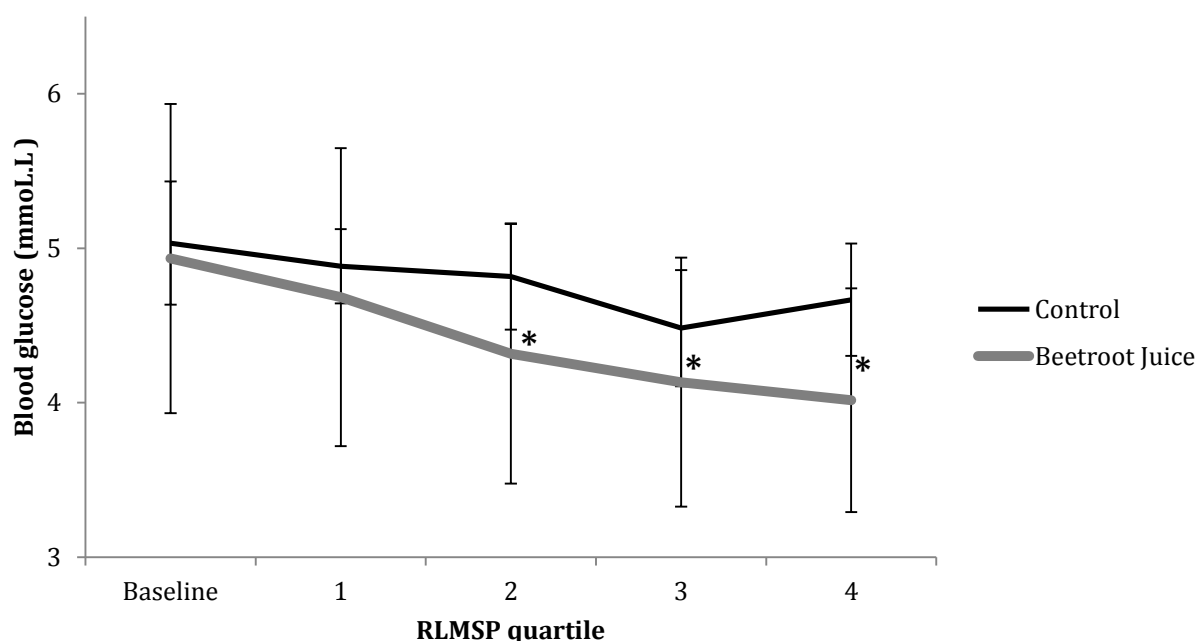


Figure 4. Changes in blood glucose levels from baseline and each quartile of the RLMSP. Data presented as means  $\pm$  standard deviations. \* denotes a significant difference to baseline ( $p < 0.05$ ).

### 3.6. Oxygen consumption

Oxygen consumption throughout the protocol was not significantly different between the two groups ( $t = 1.9$ ,  $p > 0.05$ ). Average oxygen consumption was  $35.4 \pm 5.3$  and  $30.4 \pm 1.9$  ml·kg·min<sup>-1</sup> in the control and BR (n=6) group respectively.

### 3.7. Blood lactate

ANOVA revealed significant changes in blood lactate over time ( $F_{1.98, 40} = 0.4$ ,  $p < 0.05$ ), however, no group x time interaction ( $F_{1.98, 40} = 8.1$ ,  $p > 0.05$ ) was found.

Table 2. Changes in blood lactate from baseline and after each quartile of the RLMSP. Data presented as means  $\pm$  standard deviations.

Group	Baseline	Quartile 1	Quartile 2	Quartile 3	Quartile 4
<b>Control</b> (mmol/L)	2.72 $\pm$ 2.1	5.67 $\pm$ 1.56*	4.62 $\pm$ 1.84	4.07 $\pm$ 1.73	3.78 $\pm$ 1.54
<b>Beetroot Juice</b> (mmol/L)	1.52 $\pm$ 0.54	3.55 $\pm$ 1.34*	2.45 $\pm$ 0.98*	2.33 $\pm$ 0.95	2.23 $\pm$ 0.69

\* denotes a significant difference to baseline ( $p < 0.05$ ).

### 3.8. Heart rate and RPE

RPE was significantly different over time ( $F_{3, 30} = 13.54$ ,  $p < 0.05$ ), however there was no interaction effect of RPE ( $F_{3, 30} = 0.11$ ,  $p > 0.05$ ). ANOVA revealed no group x time interaction ( $F_{3, 30} = 0.83$ ,  $p > 0.05$ ) in heart rate, however a significant effect of time was discovered ( $F_{3, 30} = 5.66$ ,  $p < 0.05$ ).

Table 3. Heart rate and RPE for each quartile of the RLMSP. Data presented as means  $\pm$  standard deviations.

RLMSP quartile	Heart rate (beats·min <sup>-1</sup> )		RPE	
	Control	Beetroot juice	Control	Beetroot juice
<b>Quartile 1</b>	172.8 $\pm$ 12.2	163.6 $\pm$ 4.51	13.17 $\pm$ 1.34	13.17 $\pm$ 0.8
<b>Quartile 2</b>	172.9 $\pm$ 11.1	164.2 $\pm$ 6.7	14.67 $\pm$ 1.49*	14.33 $\pm$ 1.74
<b>Quartile 3</b>	164.8 $\pm$ 13.7	160.5 $\pm$ 8.04	15.5 $\pm$ 0.76*	15 $\pm$ 1.17*
<b>Quartile 4</b>	164.2 $\pm$ 14.1*	159.9 $\pm$ 10.01	17.83 $\pm$ 0.69*	16.15 $\pm$ 1.62*

\* denotes significant difference to quartile 1 ( $p < 0.05$ ).

### 3.9. Serum CK

ANOVA revealed a significant time effect ( $F_{3, 27} = 8.3, p < 0.05$ ) and group x time interaction ( $F_{3, 27} = 5.093, p < 0.05$ ). CK was significantly higher following the protocol in the control group compared to the BR group (figure 4).

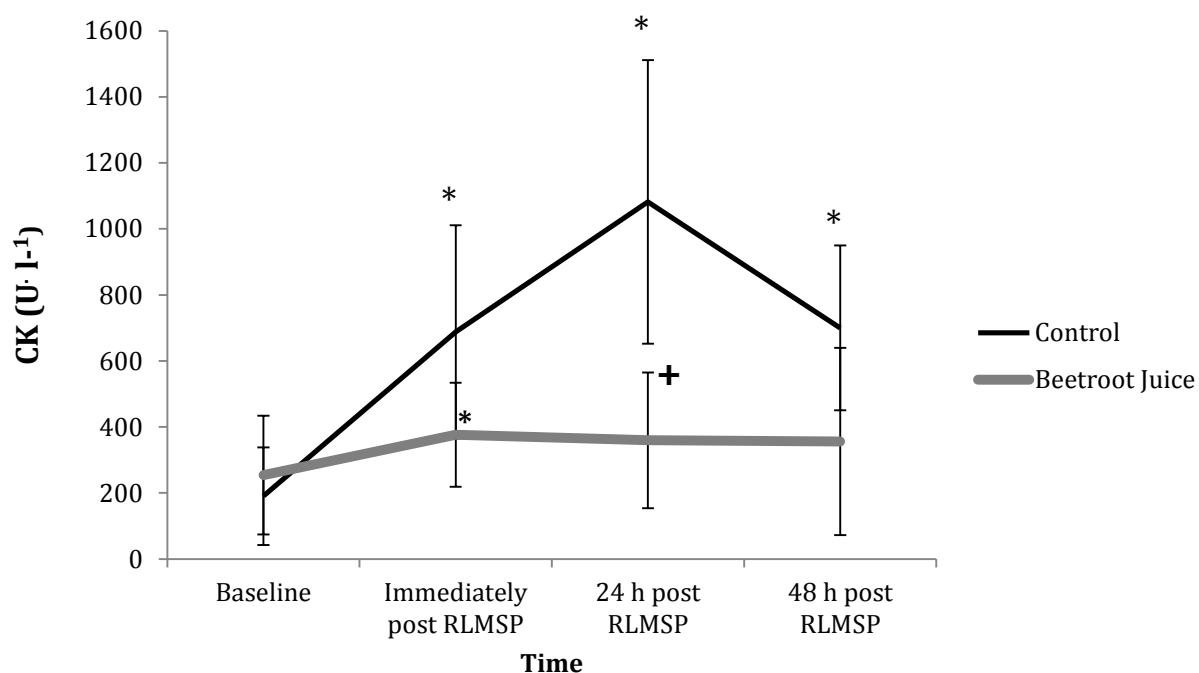


Figure 5. Changes in CK from baseline up to 48 h following the RLMSP. Data presented as means  $\pm$  standard deviations. (Control group,  $n=5$ ; BR group,  $n=6$  at 48h) \*denotes a significant difference to baseline ( $p < 0.05$ ). + denotes significant difference between groups ( $p < 0.05$ ).

### 3.10. Perceived muscle soreness

A significant time effect ( $F_{3, 30} = 27.452$ ,  $p < 0.05$ ) and group x time interaction ( $F_{3, 30} = 3.228$ ,  $p < 0.05$ ) was discovered (figure 5).

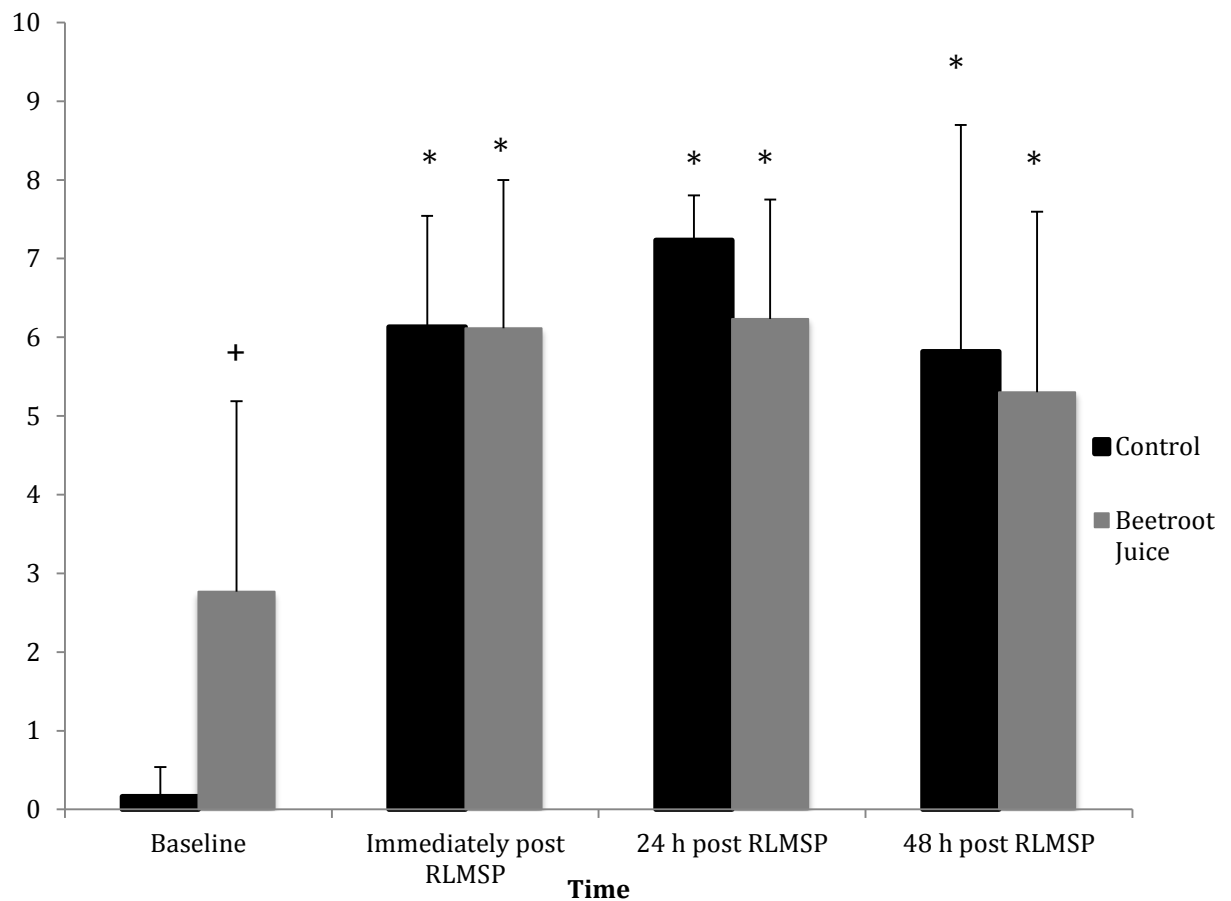


Figure 6. Changes in perceived muscular soreness up to 48 h following the RLMSP. \* denotes a significant difference to baseline ( $p < 0.05$ ). + denotes a significant difference between groups.

### 3.11. Isokinetic strength and vertical jump performance

A significant group x time interaction was discovered ( $F_{3, 27} = 3.275$ ,  $p < 0.05$ ). Peak torque was significantly reduced 24 h after the RLMSP, and was concomitantly lower than the BR group value at this time point. Peak torque in the BR group remained unchanged (figure 6).

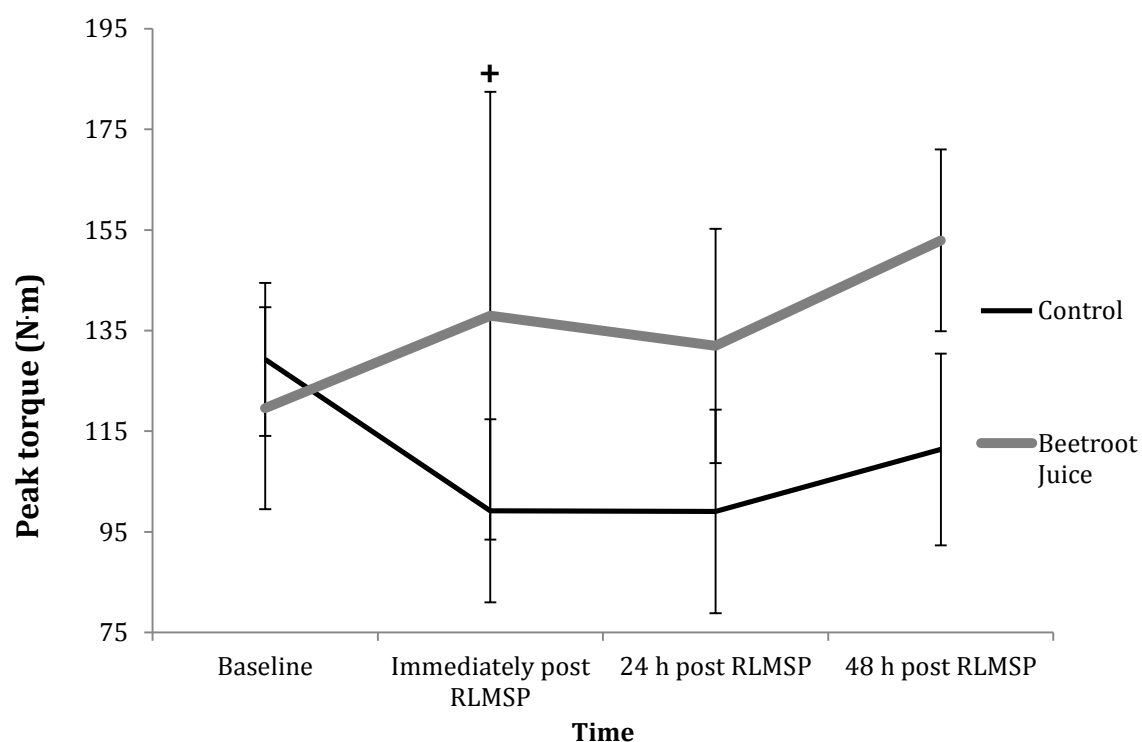


Figure 7. Isokinetic peak torque for knee flexors at  $60 \text{ deg} \cdot \text{s}^{-1}$  at baseline and following the RLMSP (Control group,  $n=5$ ; BR group,  $n=6$  at 48h). + denotes a significant difference to control group ( $p < 0.05$ ). Data expressed as means  $\pm$  standard deviations.



No group x time interaction ( $F_{3, 27} = 1.323$ ,  $p > 0.05$ ) on peak extensor torque at  $60 \text{ deg} \cdot \text{s}^{-1}$ , however, a significant time effect ( $F_{3, 27} = 5.358$ ,  $p < 0.05$ ) was evident. The groups had significantly different isokinetic peak torque immediately following the RLMSP (figure 7).

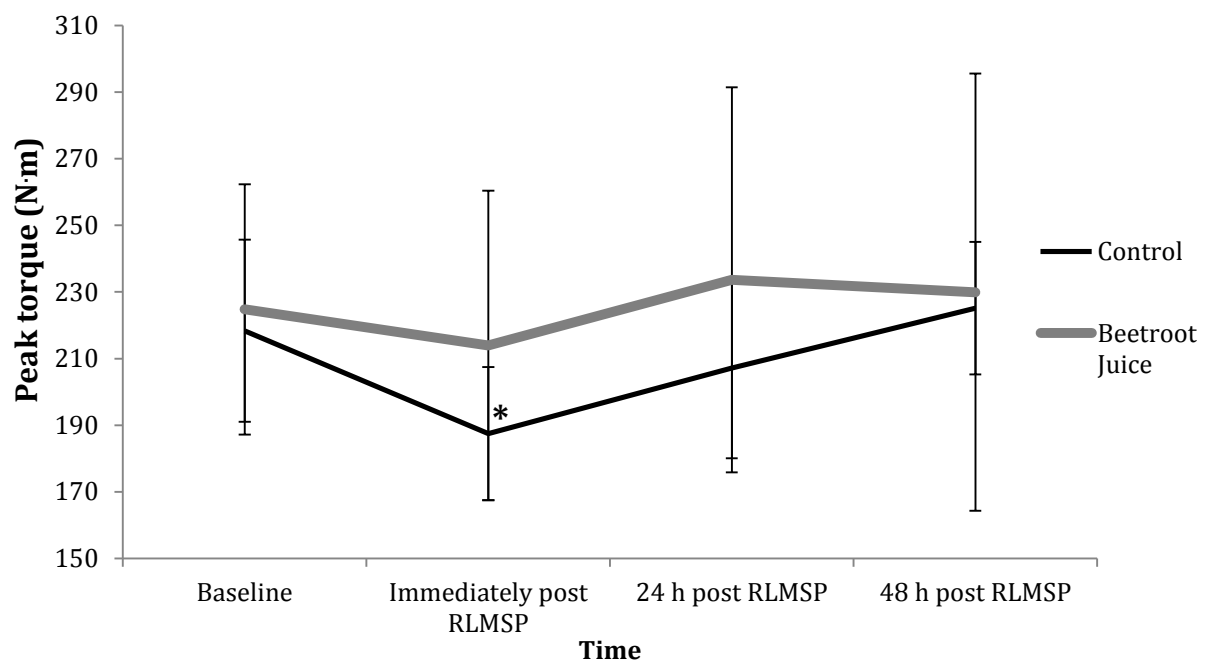


Figure 8. Isokinetic peak torque for knee extensors at  $60 \text{ deg} \cdot \text{s}^{-1}$  at baseline and following the RLMSP (Control group,  $n=5$ ; BR group,  $n=6$  at 48h). \* denotes a significant difference to baseline ( $p < 0.05$ ). Data expressed as means  $\pm$  standard deviations.

No group x time interaction ( $F_{3, 27} = 2.962$ ,  $p > 0.05$ ) was discovered for isokinetic peak torque at 240 240 deg·s<sup>-1</sup> (figure 8).

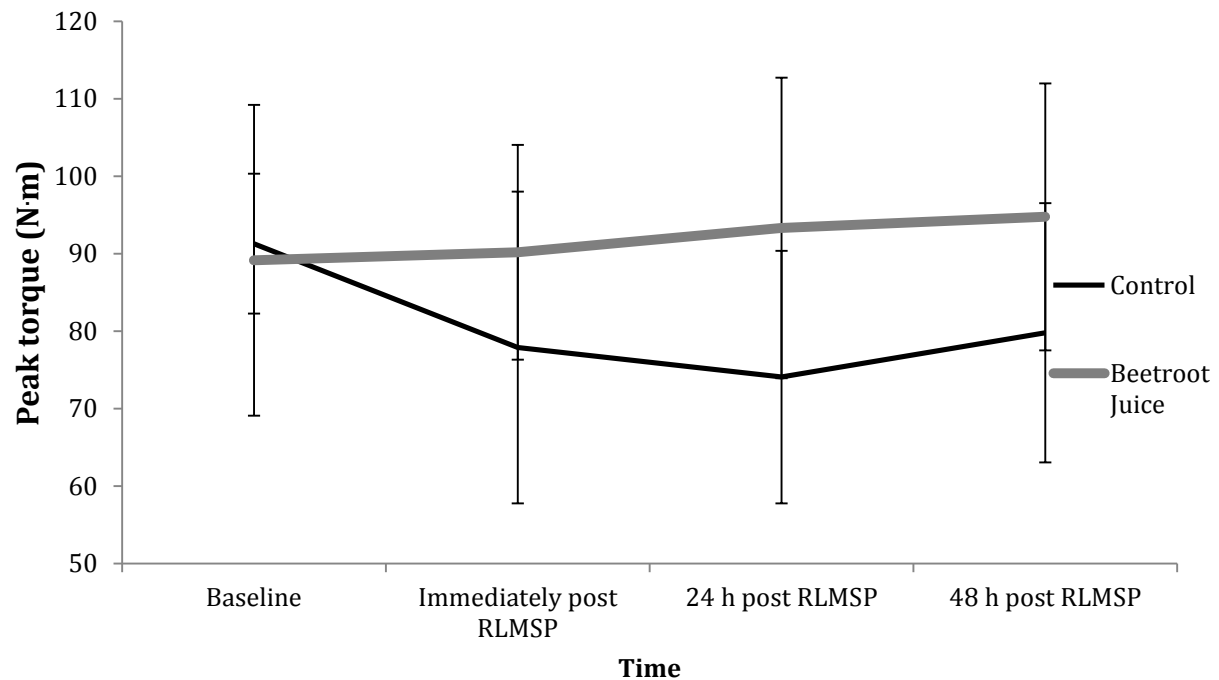


Figure 9. Isokinetic peak torque for knee flexors at 240 deg·s<sup>-1</sup> at baseline and following the RLMSP (Control group, n=5; BR group, n=6 at 48h).

No group x time interaction ( $F_{3, 27} = 1.333$ ,  $p > 0.05$ ) was discovered for peak extensor torque at  $240 \text{ deg} \cdot \text{s}^{-1}$  (figure 9).

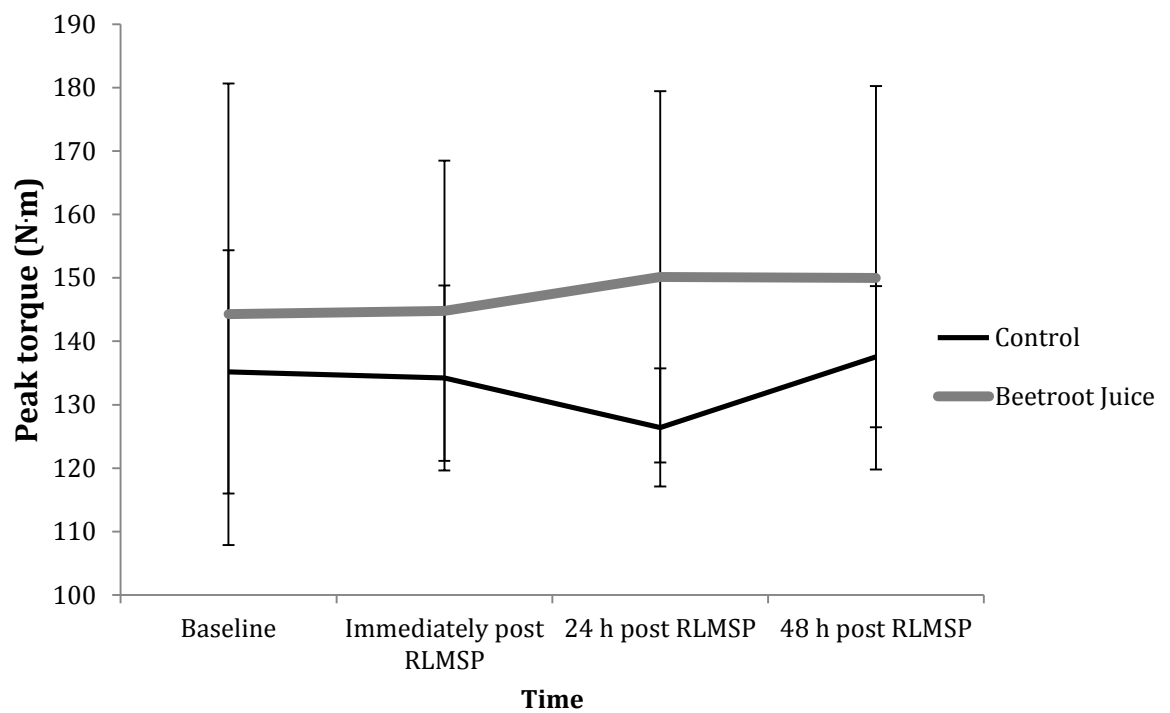


Figure 10. Isokinetic peak torque of peak knee extensor torque at  $240 \text{ deg} \cdot \text{s}^{-1}$  at baseline and following the RLMSP (Control group,  $n=5$ ; BR group,  $n=6$  at 48h). Data expressed as means  $\pm$  standard deviations.

Analysis revealed a significant time effect ( $F_{3, 30} = 6.4$ ,  $p < 0.05$ ) and group x time interaction ( $F_{3, 27} = 3.263$ ,  $p < 0.05$ ) on vertical jump performance immediately following, 24 and 48 h following the RLMSP (figure 10).

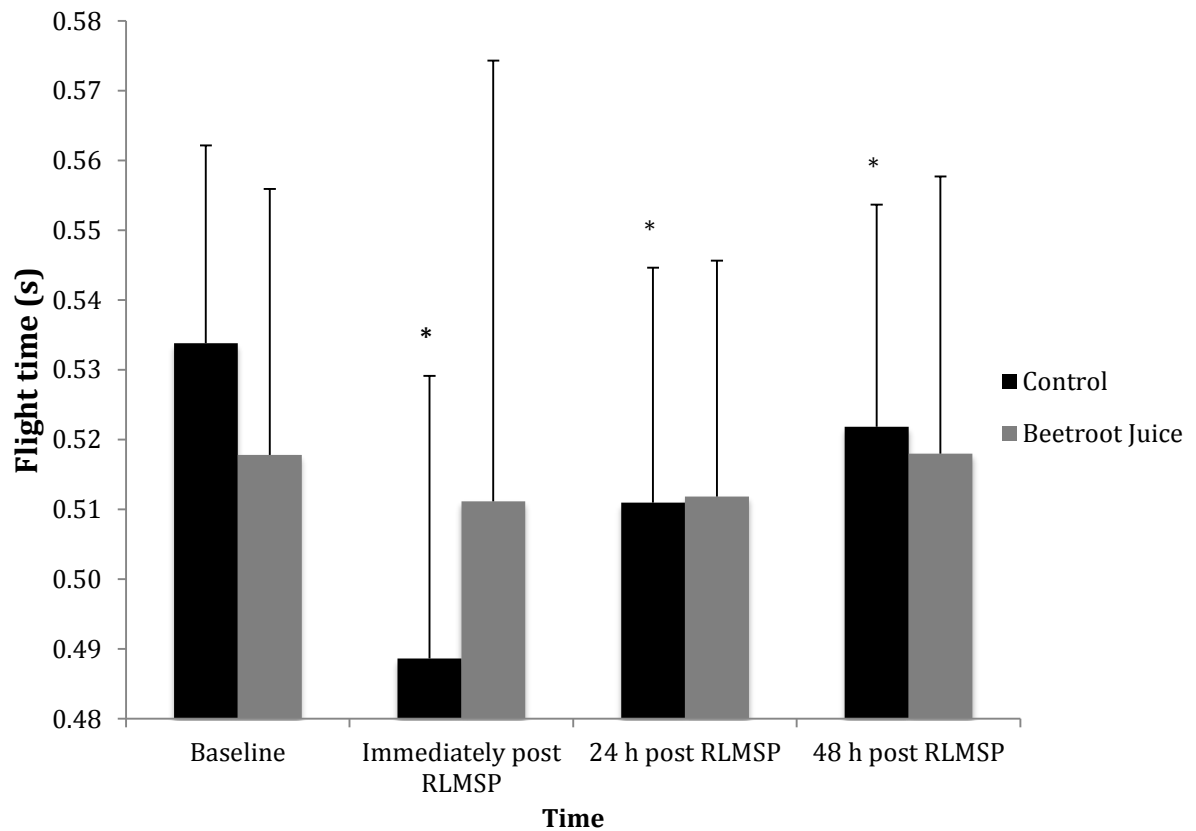


Figure 11. Changes in vertical jump performance from baseline up to 48 h following the RLMSP (Control group,  $n=5$ ; BR group,  $n=6$  at 48 h). \* denotes a significant difference to baseline ( $p < 0.05$ ). Data expressed as means  $\pm$  standard deviations.

## 4. Chapter 4 - Discussion

The principle finding of this study is that NO supplementation in the form of BR delayed the onset of fatigue during a simulated Rugby League performance, evidenced by the experimental groups preservation of relative peak sprint speed. Moreover, BR significantly improved markers of recovery and attenuated muscle damage following the RLMSP. CK, vertical jump performance and perceptual soreness were significantly improved in following BR supplementation. These findings are consistent with the experimental hypothesis, suggesting that BR may improve RLMSP performance and recovery. However, contrary to prior research, there was no significant differences in oxygen consumption (Larsen et al., 2007; Larsen et al., 2011; Vanhatalo et al., 2010a). Moreover, there were no significant differences between groups when examining isokinetic force following the RLMSP, however, the control group experienced significant force losses in the knee extensors at 60 deg·s<sup>-1</sup>. Consistent with prior literature, BR had no significant effect on physiological variables including blood lactate, heart rate and blood glucose (Wylie et al., 2012; Wilkerson et al., 2012). However, there were beneficial trends in the BR group for blood glucose and isokinetic force.

### 4.1. *Effects of BR on RLMSP performance*

Although not entirely dependent upon success, it has been reported success in intermittent sport correlates with physical fitness, the ability to complete high-intensity movements and the ability to resist fatigue (Gabbett, Kelly & Pezet, 2007; Mohr, Krstrup & Bangsbo, 2004). Wylie et al. (2013) found BR supplementation induced a 4.2% improvement Yo-YoIR1

performance; a test designed to replicate the interval and recovery periods that are representative of those encountered in team sports. Indeed, BR may also enhance rugby league specific performance in later matchplay as evidenced in this study. The experimental group's sprints in quartile 4 were not significantly different from quartile 1, however, the control group's sprints during an intermittent protocol were significantly slower (figure 3). More research is required to support this finding for elite players as there is conflicting research reporting the effects of BR supplementation in highly trained populations (Cermak et al., 2012; Bond et al., 2012; Christensen et al., 2013; Bescós et al., 2012). Furthermore, pacing strategies cannot be ruled out when explaining the differences in mean sprint speeds in quartile 4. Research on rugby league players' locomotor behavior during matches discovered an "end spurt", where running speeds increased towards the end of events (Waldron et al., 2013; Black & Gabbett, 2014).

In contrast to the experimental hypothesis, mean speed in each quartile and high speed running were not significantly different between groups, however, this may be explained by examining the supplementation protocol of this study. Comparable to this study, Wilkerson et al. (2012) found no significant effect of acute supplementation with BR (6.2 mMol NO, 2.5 h prior to performance) on cycling time-trial performance. Wilkerson et al. (2012) reported increases of plasma nitrite (a precursor to NO) concentrations following BR supplementation in five of their eight participants termed 'responders'. The increase in plasma nitrite in responders was significantly correlated with the difference in time-trial performance ( $r = -0.83$ ,  $P = 0.01$ ), suggesting that acute supplementation may be effective only in responders to

NO. Furthermore, studies using longer BR supplementation protocols have found improvements in performance (Vanhatalo et al., 2010a; Bailey et al., 2009). Cermak et al. (2012) reported a significant difference in 10 km time-trial performance following chronic BR supplementation (6 days). This study amongst others, suggests a longer (~2-6 day) supplementation protocol may be more appropriate to elicit an ergogenic effect (Wyllie et al., 2012; Cermak et al., 2012; Wilkerson et al., 2012).

#### *4.2. Effects of BR on physiological variables*

Confirming the novel finding of Wyllie et al. (2013), blood glucose was significantly reduced from baseline in the BR group during intermittent exercise. Due to the signaling properties of NO in skeletal muscle glucose uptake, it is likely glucose uptake was increased during the RLMSP following BR supplementation (Jentjens & Jeukendrop, 2003). Research has found that fatigue later in intermittent sports is a consequence of muscle glycogen and phosphocreatine depletion. It is feasible that the increased glucose uptake may have contributed to the maintenance of mean sprint speeds through the sparing of muscle glycogen, particularly in type II fibres (Tsintzas & Williams, 1998; Jentjens & Jeukendrop, 2003; Mohr, Krstrup & Bangsbo, 2004).

A consistent finding of studies examining the effects of BR is a reduction in  $\dot{V}O_2$  for a given workload i.e. improved exercise efficiency (Bailey et al., 2009; Cermak et al., 2012; Lansley et al., 2011). Upon finding no significant differences in  $\dot{V}O_2$  between groups, this study contradicts the findings of prior literature. This may be due to the aforementioned limitations when using an acute BR supplementation. In addition, technical errors with

the portable gas analyser were encountered leading to two sets of  $\dot{V}O_2$  data from the control group being lost. Indeed, the BR group's mean  $\dot{V}O_2$  was 5 L lower during the RLMSP, suggesting that BR may have had some effect on performance through an increased muscle oxygen delivery, mediated by NO vasodilation, thereby increasing oxidative metabolism and blunting intramuscular phosphocreatine decrements (Wylie et al., 2012).

#### *4.3. Effects of BR on muscle damage and recovery*

Similar to a prior study investigating fatigue and EIMD following a RLMSP, this study found significantly elevated serum CK for up to 48 h in the control group (Twist & Sykes, 2011). The experimental group experienced a significant rise in CK at 24 h following the RLMSP, however, at 24 h the control group demonstrated significantly higher serum CK concentrations. The rise in CK levels in this study, followed a similar pattern to prior research with peak serum CK found at 24 h, however, CK was higher than previously reported following a RLMSP (Twist & Sykes, 2011). This may be a result of the extended protocol, designed to induce further metabolic and mechanical EIMD (Tee, 2007). Additionally, CK appearance in the blood is reportedly dependent upon training status, with the most marked increases occurring in less trained athletes which may also explain the dissimilarities in this study (Broncaccio, Maffulli & Limongelli, 2007).

Beaton et al. (2002) discovered that antioxidant supplementation for 30 d reduced serum CK concentration at 24 h following 240 maximal eccentric contractions in humans. It is understood that an elevation in intracellular levels of calcium ions, caused by eccentric contractions, activate proteases named



calpains, which cleave muscular proteins, causing secondary damage to the muscle cell and allow the leakage of intracellular proteins such as CK (Thiebaud, 2012). Furthermore, the damaged muscle induces an influx of macrophages and neutrophils, which further promote increases in potentially damaging ROS (Pizza et al., 2002; Thiebaud, 2012; Tidball, 2005). The RLMSP is likely to have induced large increases in damaging ROS through increased oxygen usage in the mitochondria and also myofibril disruption as a result of eccentric muscular contraction (Paternelj & Coombes, 2011). While in agreement with the findings of Beaton et al. (2002), a novel finding of this study suggests that more acute antioxidant supplementation through the ingestion of BR can also attenuate muscle membrane damage and the appearance of CK. With the reported benefits of vasodilation and subsequently improved reperfusion due to NO; it is feasible the BR group would have had a greater supply of antioxidants to donate their electron, thereby neutralizing ROS and subsequently attenuating EIMD in the experimental group (Vanhatalo et al., 2010; Paternelj & Coombes, 2011).

Consistent with findings following a RLMSP and rugby league match, perceived muscular soreness was significantly higher at all time points in both groups (Twist & Sykes, 2011; Twist et al., 2013). The BR group reported significantly greater feeling of soreness at baseline, possibly due to exercise prior to the study, however, following the RLMSP there was no significant differences between groups. Perceptual soreness has a close association with myofibril disruption, suggesting the antioxidants present in BR had a protective effect on muscular structures. (Cheung, Hume & Maxwell; Paternelj & Coombes, 2011). Indeed, due to the interpretation of the soreness scale,

exaggeration/underreporting cannot be ruled out (Nielsen et al., 2009). However, an attenuated increase in perceived muscular soreness may have positive implications for improved training quality in the days following a rugby league performance (Twist & Skyes, 2011).

The pattern for isokinetic force losses in this study is inconsistent to prior literature, where neuromuscular function was disrupted for up to 24 h following a RLMSP (Twist & Sykes, 2011). Inadequate habituation to the dynamometer leading to a learning effect may explain this finding, however, the control group did experience force losses suggesting BR had an effect (figures 5-8). The control group's peak knee extensor torque at  $60 \text{ deg}\cdot\text{s}^{-1}$  was significantly lower immediately following the RLMSP whereas the BR group maintained isokinetic force. Additionally, the control group's peak isokinetic knee flexor torque at  $60 \text{ deg}\cdot\text{s}^{-1}$  was significantly lower than the BR group immediately following the RLMSP. The maintained flight time at all time points following the RLMSP in the BR group, compared to the decreases immediately, 24 and 48 h in control, adds further evidence to the theory of attenuated EIMD and maintained neuromuscular function following BR supplementation (figure 11). Folland, Maas & Jones (2000) discovered that maximal voluntary contractions *in vivo*, were enhanced in the presence of NO due improved excitation contraction coupling. Furthermore, ROS generation as a result of the RLMSP could have acted to inhibit E-C coupling in the control group by disrupting muscle ion channels and calcium release; it is feasible the antioxidants present in BR served to maintain neuromuscular function following the RLMSP by neutralizing ROS and facilitating E-C coupling (Shafat et al., 2004).

#### *4.4. Study limitations*

Pollo, Carlino & Fabrizio, (2008) found leg extension was improved 22.1% following the consumption of a placebo beverage. The authors attributed improved performance to cue signaling acting to restrict the central governor motor response. Due to 11 of the 12 participants correctly identifying their beverage, the placebo effect cannot be ruled out when examining the positive effects of BR in this study. A further weakness of this study is that plasma nitrite/NO was not measured, making identification of responders and non-responders impossible (Wilkerson et al., 2012). A final weakness of this study is a lack of control over the participants diet and prior activity. Although the food and activity diary method is simple and facilitates ease of adherence, the reliability of data can range with under-reporting and diet modifications to appear more socially desirable is common (Deakin, 2006). Therefore it can only be presumed participants adhered to the dietary regimen required in this study and the benefits seen were a result of BR supplementation.

#### *4.5. Practical applications*

The findings of this study, in conjunction with Wylie et al. (2013), suggest acute BR supplementation may be ergogenic for intermittent exercise. This may prove practically important as chronic supplementation with BR may become quite invasive for athletes due to adherence, the consumption of large boluses of BR and side effects such as beeturia (Bailey et al., 2009; Vanhatalo et al., 2010a). Additionally, it would appear BR supplementation before and up to 48 h following (~13 mMol a day; total antioxidant capacity of 697.9 umol) rugby league specific exercise is beneficial in protecting and

promoting recovery from EIMD. This may have particular benefits for athletes who are required to recover for training and/or matches within 48h. However, further research is required into the effects of BR on performance and the attenuation of EIMD in elite rugby league athletes.

#### *4.6. Conclusion*

In conclusion, acute BR supplementation facilitated the maintenance of peak sprint speeds during the final quartile of the RLMSP. This finding may have practical significance as it could allow for improved performance later in a match. The improvements in performance may have been induced by increased glucose uptake into the muscle. Finally, BR supplementation (13 mMol a day) may act to prevent and attenuate EIMD following the RLMSP as evidenced by significantly reduced CK, perceived muscular soreness and maintained neuromuscular function. More research is required to examine the effects of BR supplementation in elite rugby league players before these findings can be applied in a professional setting.

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## **6. Appendix**

Appendix 1. Ethical approval.

Appendix 2. Foods rich in dietary nitrate.

Appendix 3. Foods with high antioxidant content.

Appendix 4. Food diary

Appendix 5. Dietary recommendation.

Appendix 6. Post-RLMSP snack.

Appendix 7. Participant information sheet.

Appendix 8. Risk assessment.

Appendix 9. Informed consent form.

Appendix 10. Pre-test health questionnaire.

Appendix 11. Raw data. (Attached via USB stick).

Appendix 12. SPSS data. (Attached via USB stick).

Appendix 13. SPSS output. (Attached via USB stick).

Appendix 14. Completed pre-test health questionnaires and informed consent forms

## Appendix 15. G\*Power calculation.

**Appendix 1**

*Faculty of Life Sciences*  
*Research Ethics Committee*

frec@chester.ac.uk

15th May 2014

Dear Casey,

**Study title:**        **The effects of acute beetroot juice supplementation on the physiological responses to a rugby league match simulation protocol and recovery.**

**FREC reference:**    **895/14/CW/SES**

**Version number:**   **1**

Thank you for sending your application to the Faculty of Life Sciences Research Ethics Committee for review.

I am pleased to confirm ethical approval for the above research, provided that you comply with the conditions set out in the attached document, and adhere to the processes described in your application form and supporting documentation.

The final list of documents reviewed and approved by the Committee is as follows:

<b>Document</b>	<b>Version</b>	<b>Date</b>
Application Form	1	March 2014
Appendix 1 – List of References	1	March 2014
Appendix 2 – C.V. for Lead Researcher	1	March 2014
Appendix 3 – Participant Information Sheet	1	March 2014
Appendix 4 – Participant Consent Form	1	March 2014
Appendix 5 – Risk Assessment Form	1	March 2014
Appendix 6 – Pre-test Health Questionnaire	1	March 2014
Appendix 7 – Food Diary	1	March 2014
Appendix 8 – Nitrate content of foods	1	March 2014
Appendix 9 – Antioxidant content of foods	1	March 2014
Appendix 10 – Study methods	1	March 2014
Appendix 11 – Written Permission, University of Chester Human Performance/Exercise Physiology Research Laboratories	1	March 2014
Response to FREC request for further information or clarification		May 2014
Appendix 3 – Participant Information Sheet	2	May 2014
Appendix 4 – Participant Consent Form	2	May 2014
Appendix 8 – Nitrate content of foods	2	May 2014
Appendix 9 – Antioxidant content of foods	2	May 2014
Appendix 12 – Example of daily dietary intake	1	May 2014
Appendix 13 – Example of a post-exercise snack	1	May 2014
Appendix 14 – Written permission from the	1	May 2014

University of Chester Men's Rugby Coach, Gareth Evans.		
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Please note that this approval is given in accordance with the requirements of English law only. For research taking place wholly or partly within other jurisdictions (including Wales, Scotland and Northern Ireland), you should seek further advice from the Committee Chair / Secretary or the Research and Knowledge Transfer Office and may need additional approval from the appropriate agencies in the country (or countries) in which the research will take place.

With the Committee's best wishes for the success of this project.

Yours sincerely,

**Dr. Stephen Fallows**

Chair, Faculty Research Ethics Committee

Enclosures: Standard conditions of approval.

Cc. Supervisor/FREC Representative

## Appendix 2

### Nitrate content of foods

Please try to restrict the following meats & vegetables, particularly those with high-very high nitrate content:

Processed and smoked meats including;

Bacon

Hot-dogs

Luncheon meat

SPAM

Ham

Bologna

Salami

Peperoni

corned beef

Note; try to eat fresh meats and fish such as chicken, beef & salmon

### Classification of vegetables according to nitrate content'

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Table retrieved from Hord, N., Tang, Y., & Bryan, N. (2009). Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *American Journal of Clinical Nutrition*, 90(1), 1-10.



**Appendix 3**

Foods high in total antioxidant capacity according to Pellegrini et al. (2003).

Please remove these foods (particularly the foods with a high rank e.g.

1,2,3,4...) as best possible in the days leading up to and during the testing phase of this study.

Vegetables

Antioxidant content rank

Beverages

Antioxidant content rank

Other beverages

Antioxidant content rank

8  
0  
1  
4  
3  
2  
9  
1  
1  
9  
8  
0  
7  
6  
5  
7  
1  
2  
4  
3  
6  
5

Fruit

Antioxidant content rank

|

|

|

Oils

Antioxidant content rank

|

|

|

## Appendix 4

# Food Diary

**Name:**

**Date:**

/ /2014

<u>Breakfast</u> - Food Consumed	Serving size	Time	Cooking method	Comments

<u>Lunch</u> - Food Consumed	Serving size	Time	Cooking method	Comments

<u>Dinner</u> - Food Consumed	Serving size	Time	Cooking method	Comments

<u>Snacks</u> - Food Consumed	Serving size	Time	Cooking method	Comments

## Appendix 5

### Daly dietary intake example:

To work out the recommended dietary intake for this study please follow the instructions below.

**Body weight (kg) x Recommended nutrient intake ( $\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ) = Total recommended nutrient intake.**

**Practical example for carbohydrates:**  $60 \times 5 = 300$  g total carbohydrate, protein  $\times 1.5 = 90$  g total protein.

To monitor your nutrient intake I recommend you use an online food diary application such as livestrong (myplate) or myfitnesspal, both downloadable free of charge for iPhones and Androids.

Please see the example of a diet consisting of 300 g carbohydrate.

Breakfast:

40 g Quaker Oats with 300ml semi skimmed milk = 38 g carbohydrate, 11.2 g protein, 6.6 g fat.

8 oz Tropicana orange juice = 26 g carbohydrate, 2 g protein.

Lunch:

150 g penne pasta = 132 g carbohydrate

50 g chopped tomato = 2 g carbohydrate

100 g skinless roast chicken breast = 1 g carbohydrate, 23 g protein.

Dinner:

450 g chinese chicken curry and rice = 83.5 g carbohydrate, 30 g protein.

Snacks: 1 banana = 18 g carbohydrate, 1 g.

## Appendix 6

### Example of a post-exercise snack following visit 2.

Individual example: Body weight = 60 kg.

$60 \text{ kg} \times 0.3 \text{ g} \cdot \text{kg}^{-1} \text{ body weight protein} = 18 \text{ g protein required.}$

$60 \text{ kg} \times 1 \text{ g} \cdot \text{kg}^{-1} \text{ body weight carbohydrate} = 60 \text{ g carbohydrate required.}$

Examples of snacks:

500 ml Yazoo Chocolate Milkshake = 60 g carbohydrate, 18g protein.

60 g grilled chicken breast & 80 g wholemeal penne pasta = 57 g carbohydrate, 20 g protein.

90 g PowerBar Recovery drink with water = 63 g carbohydrate, 18 g protein.

Please do not hesitate to ask for an example of a snack relative to your body weight.



## Appendix 7



### Participant information sheet

#### **The effects of acute beetroot juice supplementation on the physiological responses of a rugby league match simulation protocol and recovery**

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

#### **What is the purpose of the study?**

The purpose of this study is to investigate the effect of acute beetroot juice supplementation on the physiological responses and recovery from a Rugby League match simulation protocol. Beetroot juice supplementation has exhibited an uptake in consumption due to research showing potential performance enhancement during endurance, high-intensity and intermittent exercise. However, there is little research on team sport exercise with previous research in the area having neglected to examine the efficacy of beetroot juice supplementation on recovery.

#### **Why have I been chosen?**

You have been chosen because you are a University level Rugby League athlete who regularly takes part in competitive matches.

**Do I have to take part?**

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect you in any way.

**What will happen to me if I take part?**

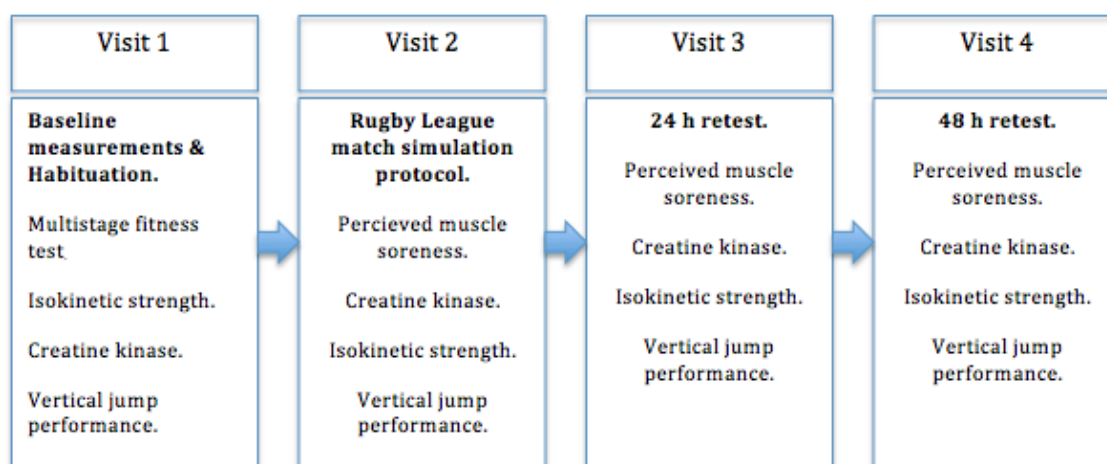
If you decide to take part, you will be given this information sheet to keep and asked to sign a consent form. You will be required to attend a total of four sessions; visits 1 and 2 will last no longer than 2.5 hrs and visit 3 and 4 lasting no longer than 1 h. You will be required to complete a 10-day food diary while avoiding foods high in dietary nitrate and rich in antioxidants (lists provided) and antibacterial mouthwash during the testing phase. You will also be required to abstain from alcohol in the 24 hr prior to testing. In addition, you will be required to have a moderate to high carbohydrate intake (60–70% of energy consumed, ~5–7 grams per kilogram of your body weight), a protein consumption of ~1.5 grams per kilogram of your body weight, and a fat ingestion of ~10–20% of whole energy consumption (an example is available on request). You will also be requested to sustain an elevated carbohydrate (1 gram per kilogram of your body weight) and protein (0.3 grams per kilogram of your body weight) consumption in the 2 h following the rugby league match simulation protocol in visit 2 (an example is available on request). It is recommended you use a food diary app such as livestrong (Myplate) or myfitnesspal, which will provide you with your nutrient intake and content of the foods you eat.

On your first visit you will be required to perform a series of baseline measurements including isokinetic strength, creatine kinase levels, vertical jump performance, multistage fitness test to exhaustion to predict your  $VO_{2max}$  and a 10 minute familiarization to the Rugby League match simulation protocol. On your second visit you will be asked to arrive fasted overnight at



09:00 having consumed a 70 ml Beet-it shot or placebo drink before completing a 92.08 min min rugby league match simulation protocol. You will then complete measures of isokinetic strength, creatine kinase activity, perceived muscular soreness, vertical jump performance as well as providing finger tip blood samples. You will also be required to consume a further 70 ml Beet-it shot, 6 hrs after the consumption of your first beetroot juice drink. You will be required to consume a 2 x Beet-it (separate by 6 hrs) shots a day for 2 more days. During the final two visits, measures of isokinetic strength, creatine kinase activity, perceived muscular soreness and vertical jump performance will be retested. During the study you may experience symptoms of beeturia (red urine), however, this is a natural symptom following beetroot juice consumption and is not harmful.

Testing can be flexible to suit you, however, please note that the final three testing sessions must be separated by 24 h each with a one-week recovery between visit 1 & 2.



**Figure 1:** Schematic of study design.

### What are the possible disadvantages and risks of taking part?

Muscle soreness following visit 2 may be a disadvantage of taking part in this study. Reduced range of motion is a further symptom of muscle damage which you may experience following visit 2, however, muscle soreness and reduced range of motion are common following exercise lasting usually 48-hrs.

You will also be required to abstain from foods high in nitrates and antioxidants, which may affect your existing dietary intake.

**What are the possible benefits of taking part?**

On completion of this research, participants will receive a predicted  $VO_{2max}$  score. This may function as a valuable resource when planning your training programmes. Should the effects of beetroot juice prove beneficial to performance and recovery, you may confidently use beetroot juice as an ergogenic aid to your performance.

**What if something goes wrong?**

If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, please contact Professor Sarah Andrew, Dean of the Faculty of Life Sciences, University of Chester, Parkgate Road, Chester, CH1 4BJ, 01244 513055.

**Will my taking part in the study be kept confidential?**

All information which is collected about you during the course of the research will be kept strictly confidential so that only the researcher carrying out the research will have access to such information.

**What will happen to the results of the research study?**

The results will be written up into a dissertation for my final project of my MSc. Individuals who participate will not be identified in any subsequent report or publication.

**Who is organising the research?**

The research is conducted as part of a MSc in Sport & Exercise Physiology within the Department of Sport & Exercise Sciences at the University of Chester. The study is organised with supervision from the department, by Casey Walker an MSc student.

**Who may I contact for further information?**

If you would like more information about the research before you decide whether or not you would be willing to take part, please contact:

**Thank you for your interest in this research**

## Appendix 8 - Risk Assessment Form

*Please consult a technician for advice before completing this form*

<b>HAZARD</b> (e.g. equipment / procedure / environment / etc.)	<b>PERSONS AT RISK</b>	<b>RISK OF:-</b>	<b>CONTROLS TO BE FOLLOWED</b>
BIODEX ISOKINETIC DYNAMOMETER	experimental participants	joint sprains, muscle strains/tears	pre-test health questionnaire to check for existing injuries, ensure adequate warm-up, ensure correct lim alignment
BLOOD SAMPLING (CAPILLARY)	experimental participants/ experimenter/technician	blood-borne infection (e.g. HIV, hepatitis), bacterial infection, discomfort (repeated skin puncturing), fainting/dizziness/nausea	blood testing guidelines printed in lab manuals and blood analysis area, experimenters trained in lab practicals and tutorials with technicians, ask participants if they are prone to fainting during blood tests, take first sample while subject seated, follow blood-testing guidelines
CYCLE ERGOMETER WEIGHTS	staff /students	back injury from moving heavy weights container	do not lift full container, push container if possible, use correct lifting technique.
DIETARY SUPPLEMENTATION	experimental participants	Dependant on supplement. See CAFFEINE or GLUCOSE SUPPLEMENTATION entries or seek advice from study supervisor for other substances.	dosage advised by supervisor, individual supplementation doses checked by 2 people.
EXERCISE TO EXHAUSTION	experimental participants	breathlessness, dizziness, nausea, falls, cardiac arrest	pre-test health questionnaire, test not suitable for untrained participants, ensure subject familiarised with test ergometer, tests to be supervised by technician/tutor/suitably experienced PhD student.
GAS ANALYSIS (douglas bag collection / servomex gas analyser / harvard	experimental participants	respiratory infection, claustrophobic feelings	use sterilised mask and 3-way valve for each subject, check subject not feeling claustrophobic, ensure tubing attached to expired air port of 3-way valve, experimenter to wash hands after handling used mask/valve.

dry gas meter)			
GPS SYSTEMS e.g. GPSports SPI Elite	experimental participants	Discomfort, bruising if participant falls onto body area where GPS unit is worn.	wear supplied vests with padded pocket for GPS unit.
HEART RATE MONITORS	experimental participants	skin infection	wash chest belts after use
HOLTAIN WALL-MOUNTED STADIOMETER	experimental participants	bangs on the head	bring measuring board down slowly onto subject's head
JUMP TESTS	experimental participants	muscle strains/tears	pre-test health questionnaire, ensure adequate warm-up, supervise test and correct poor technique
LACTATE PRO	see BLOOD SAMPLING (CAPILLARY)		
MAXIMAL AEROBIC CAPACITY TESTS	experimental participants	breathlessness, dizziness, nausea, falls, cardiac arrest	pre-test health questionnaire, test not suitable for untrained participants, ensure subject familiarised with test ergometer, test to be supervised by technician/tutor.
OPTOJUMP TIMING SYSTEM	experimental participants	tripping over timing gates/wires	ensure gates set wide enough for running/jumping activity, keep wires out of the way or mark with cones
OXYCON ONLINE GAS ANALYSER	experimental participants/ experimenter	respiratory infection, claustrophobic feelings	use sterilised mask and turbine for each subject, check subject not feeling claustrophobic, experimenter to wash hands after handling used mask/turbine
RUNNING TESTS (in the field) / MULTI-STAGE FITNESS TEST (BLEEP TEST)	experimental participants	cardiac arrest in at-risk participants, muscle strains, joint sprains	pre-test health questionnaire, test suitable for young/fit/healthy participants, ensure running surface is suitable and participants have enough space to complete test without colliding.
SPHYGMOMANOMETER & STETHOSCOPE	experimental participants/ experimenter	discomfort from over-inflated cuff, ear infection from use of stethoscope	inflate to 160/170mmHg for healthy participants, reduce cuff pressure as quickly as possible once reading taken, clean stethoscope earpieces with alcohol wipes and allow to dry before use.
STABILOMETER (BIODEX)	experimental participants	soft tissue injuries / bone breaks from falls (in some participants)	supervision by technician / tutor / post-graduate student for elderly/infirm/balance-impaired participants

ARTIFICIAL 3G PLAYING SURFACE	Experimental participants/ experimenter	Slips, trips, hard surface (if iced over)	New Surface layed October 2011. Pitch is inspected before every training session and every weekday morning by Sport and Rec staff/ Grounds and Gardens staff. Users are aware to report any damage to Sport and Rec staff. The pitch is brushed weekly. If the weather has hardened the surface, users will be informed that the pitch is unplayable
FOOTWEAR FOR ARTIFICIAL 3G PLAYING SURFACE	Participants	Trips, slips, sprains	Unable to turn as well in long length stud/ blades and they are therefore banned. Moulded studs or AstroTurf specific shoes recommended
JAMES WHITE BEET IT SPORTS SHOT	Experiental participants.	Beeturia (red urine).	Beeturia poses no risk the to health of participants, nonetheless participants will be warned they may experience symptoms of beeturia such as red urine during the supplementation period.

Experimenter  
Signature

Technician  
Signature

Date

**Appendix 9****INFORMED CONSENT FORM**

**Title of Project: The effects of acute beetroot juice supplementation on the physiological responses of a rugby league match simulation protocol and recovery.**

**Name of Researcher: Casey Walker**

Please tick the box if you agree with the statement:

I confirm that I have read and understood the participant information sheet for the above-named study, and have had the opportunity to ask the lead researcher any questions.

☐

I understand that my participation is voluntary, and that I am free to withdraw from participating in the study at any time, without giving any reason and without my rights being affected.

☐

I agree to take part in the above study.

☐

---

Name of Participant

Date

Signature

---

Name of Researcher

Date

Signature

*(1 for participant; 1 for researcher)*

## PRE-TEST HEALTH QUESTIONNAIRE

(PLEASE NOTE THAT THIS INFORMATION WILL BE CONFIDENTIAL)

Name:..... DOB:..... Age:.....

### **The effects of acute beetroot juice supplementation on the physiological responses of a rugby league match simulation protocol and recovery.**

Please answer these questions truthfully and completely. The purpose of this questionnaire is to ensure that you are fit and healthy enough to participate in this laboratory practical/research project.

- |  | <b>Yes</b>               | <b>No</b>                |
|--|--------------------------|--------------------------|
| 1. Have you in the past suffered from a serious illness or accident.<br>If Yes, please provide details | <input type="checkbox"/> | <input type="checkbox"/> |

.....  
 .....  
 .....

- |   | <b>Yes</b>               | <b>No</b>                |
|---|--------------------------|--------------------------|
| 2. Have you consulted your doctor the last 6 months<br>If Yes, please provide details | <input type="checkbox"/> | <input type="checkbox"/> |

.....  
 .....  
 .....

3. Do you suffer, or have you suffered from:
- |  | <b>Yes</b> | <b>No</b> |
|--|------------|-----------|
|--|------------|-----------|

Asthma	<input type="checkbox"/>	<input type="checkbox"/>
--------	--------------------------	--------------------------



Diabetes	<input type="checkbox"/>	<input type="checkbox"/>
Bronchitis	<input type="checkbox"/>	<input type="checkbox"/>
Epilepsy	<input type="checkbox"/>	<input type="checkbox"/>

High blood pressure	<input type="checkbox"/>	<input type="checkbox"/>
---------------------	--------------------------	--------------------------

<b>Yes</b>	<b>No</b>
------------	-----------

4. Is there any history of heart disease in your family

<input type="checkbox"/>	<input type="checkbox"/>
--------------------------	--------------------------

<b>Yes</b>	<b>No</b>
------------	-----------

5. Are you suffering from any infectious skin diseases, sores, blood wounds, or infections i.e., Hepatitis B, HIV, etc.?

<input type="checkbox"/>	<input type="checkbox"/>
--------------------------	--------------------------

If Yes, please provide brief details

.....

.....

<b>Yes</b>	<b>No</b>
------------	-----------

6. Are you currently taking any medication

<input type="checkbox"/>	<input type="checkbox"/>
--------------------------	--------------------------

If Yes, please provide details

.....

.....

.....

<b>Yes</b>	<b>No</b>
------------	-----------

7. Are you suffering from a disease that inhibits the sweating process

<input type="checkbox"/>	<input type="checkbox"/>
--------------------------	--------------------------

<b>Yes</b>	<b>No</b>
------------	-----------

8. Is there anything to your knowledge that may prevent you from participating in the testing that has been outlined to you?

<input type="checkbox"/>	<input type="checkbox"/>
--------------------------	--------------------------

If Yes, please provide details

.....

.....

.....

#### Your Recent Condition

<b>Yes</b>	<b>No</b>
------------	-----------

- Have you eaten in the last 2 hours?

<input type="checkbox"/>	<input type="checkbox"/>
--------------------------	--------------------------

If Yes, please provide details

.....  
 .....

- Have you consumed alcohol in the last 24hr ☐ ☐
  - Evaluate your diet over the last two days. **Poor Average Good Excellent**
  - Have you had any kind of illness or infection in the last 2 weeks ☐ ☐
  - Have you exercised in the last 2 days? ☐ ☐
- If Yes, please describe below

.....  
 .....  
 .....

Persons will not be permitted to take part in any experimental testing if they:

- have a known history of medical disorders (i.e. hypertension, heart or lung disease)
- have a fever, suffer from fainting or dizzy spells
- are currently unable to train because of a joint or muscle injury
- have had any thermoregulatory disorder
- have gastrointestinal disorder
- have a history of infectious diseases (i.e. HIV or Hepatitis B)
- have, if pertinent to the study, a known history of rectal bleeding, anal fissures, haemorrhoids or any other similar rectal disorder.

My responses to the above questions are true to the best of my knowledge and I am assured that they will be held in the strictest confidence.

Name: (Participant)..... Date:.....

Signed (Participant): .....

Name: (Researcher)..... Date:.....

Signed (Researcher): .....

**Appendix 15****G\*Power calculation.**

A moderate effect size of 0.6 was selected with a power of 80% and significance level of 0.05.